

(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT & TRADEMARK OFFICE

**TRANSMITTAL LETTER TO THE
UNITED STATES
DESIGNATED/ELECTED OFFICE
(DO/EO/US) CONCERNING A FILING
UNDER 35 U.S.C. 371**

ATTORNEY'S DOCKET NUMBER
109362U.S. APPLICATION NO.
(if known, sec 37 C.F.R. 1.5)

09/830457

INTERNATIONAL APPLICATION NO.
PCT/IL99/00570INTERNATIONAL FILING DATE
October 27, 1999PRIORITY DATE CLAIMED
October 27, 1998TITLE OF INVENTION
METHOD FOR GOLD DEPOSITIONAPPLICANTS FOR DO/EO/US
Erez BRAUN, Yoav EICHEN, Uri SIVAN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
- ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
- ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
- ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
- ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☒ Entitlement to small entity status is hereby asserted.
16. ☒ Other items or information: Submission of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

U.S. APPLIC/ C.F.R. 1.5) 09/830457	INTERNATIONAL APPLICATION NO. PCT/IL99/00570	ATTORNEY'S DOCKET NUMBER 109362
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17. <input checked="" type="checkbox"/> The following fees are submitted: Basic National fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO\$860.00 International preliminary examination fee paid to USPTO (37 CFR 1.482)\$690.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))\$710.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO\$1,000.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)\$ 100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =	CALCULATIONS	PTO USE ONLY																																				
Surcharge of \$130.00 for furnishing the oath or declaration later than <input checked="" type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	\$860.00																																					
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 15%;">Claims</th> <th style="width: 15%;">Number Filed</th> <th style="width: 10%;">Number Extra</th> <th style="width: 10%;">Rate</th> </tr> </thead> <tbody> <tr> <td>Total Claims</td> <td>42- 20 =</td> <td>22</td> <td>X \$ 18.00</td> </tr> <tr> <td>Independent Claims</td> <td>3- 3 =</td> <td>0</td> <td>X \$ 80.00</td> </tr> <tr> <td colspan="3">Multiple dependent claim(s)(if applicable)</td> <td>+ \$270.00</td> </tr> <tr> <td colspan="3" style="text-align: right;">TOTAL OF ABOVE CALCULATIONS =</td> <td>\$1,256.00</td> </tr> <tr> <td colspan="3">Reduction by 1/2 for filing by small entity, if applicable.</td> <td>- \$628.00</td> </tr> <tr> <td colspan="3" style="text-align: right;">SUBTOTAL =</td> <td>\$628.00</td> </tr> <tr> <td colspan="3">Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR 1.492(f)).</td> <td>+ \$</td> </tr> <tr> <td colspan="3" style="text-align: right;">TOTAL NATIONAL FEE =</td> <td>\$628.00</td> </tr> </tbody> </table>	Claims	Number Filed	Number Extra	Rate	Total Claims	42- 20 =	22	X \$ 18.00	Independent Claims	3- 3 =	0	X \$ 80.00	Multiple dependent claim(s)(if applicable)			+ \$270.00	TOTAL OF ABOVE CALCULATIONS =			\$1,256.00	Reduction by 1/2 for filing by small entity, if applicable.			- \$628.00	SUBTOTAL =			\$628.00	Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 month from the earliest claimed priority date (37 CFR 1.492(f)).			+ \$	TOTAL NATIONAL FEE =			\$628.00	\$	
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a. ☒ Check No. 118531 in the amount of \$628.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 15-0461. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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 REGISTRATION NUMBER: 30,411

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Erez BRAUN, Yoav EICHEN, Uri SIVAN

Application No.: U.S. National Stage
of PCT/IL99/00570

Filed: April 27, 2001

Docket No.: 109362

For: METHOD FOR GOLD DEPOSITION

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

Please cancel claims 1-41 without prejudice to or disclaimer of the subject matter
contained therein.

Please add new claims 42-83 as follows:

REMARKS

Claims 42-82 are pending. By this Preliminary Amendment, claims 1-41 are cancelled and claims 42-82 are added. Prompt and favorable examination on the merits is respectfully solicited.

Respectfully submitted,



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Registration No. 27,075

Thomas J. Pardini
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JAO:TJP/cmm

Date: April 27, 2001

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<p>DEPOSIT ACCOUNT USE AUTHORIZATION Please grant any extension necessary for entry; Charge any fee due to our Deposit Account No. 15-0461</p>
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- - 42. A method for depositing gold at one or more sites on a substrate, comprising:

(a) binding, depositing or forming nucleation centers at said one or more sites; said nucleation centers comprising at least one member of a recognition group consisting of two or more {}molecules or complexes which bind {}to each other, the other member including or forming said one or more sites, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms, a metal-containing complex and molecules.

(b) Contacting under appropriate conditions said one or more sites with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is essentially not deposited on the substrate unless a nucleation center is present on the substrate and in the presence of a nucleation center at said one or more sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said one or more sites.: - -

- - 43. A method according to Claim 42, wherein the one member of the recognition group is coupled to at least one nucleation center, being one or more of the group consisting of: cluster containing metal atoms and metal containing complexes and metals. - -

- - 44. A method according to Claim 42, wherein the one member of the recognition group is coupled to at least one nucleation center being one or more of the group consisting of gold particle, cluster containing gold atoms and gold-containing complexes and molecules. - -

45. A method according to Claim 42, wherein the one member of the recognition group is coupled to at least one nucleation center being one or more of the group consisting of cluster containing gold atoms and gold-containing complexes and molecules. - -
46. A method according to Claim 42, wherein said {} recognition group is a member of the group consisting of: an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain. - -
47. A method according to Claim 42, wherein said treatment composition is an aqueous solution. - -
48. A method according to Claim 47, wherein said gold-providing agent is $\text{Au}^+(\text{SCN})_2^-$. - -
49. A method according to Claim 47, wherein said reagent is hydroquinone or naphthohydroquinone. - -
50. A method for assaying presence of a specific substance at sites on a substrate, comprising:
- (a) providing conditions allowing formation of nucleation centers on sites containing said substance;
 - (b) contacting said substrate with a treatment composition comprising a soluble gold providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(c) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating presence of said substance at said site. - -

- 51. A method according to Claim 51, wherein step (a) comprises:

(a1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said substance, bound to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms and a metal-containing complex; and

(a2) contacting said substrate with said agents. - -

-- 52. A method according to Claim 51, wherein said moiety is included in one member of a recognition couple consisting of two molecules or complexes which specifically bind to one another, the other member included in or constituting said substance. - -

-- 53. A method according to Claim 52, wherein said recognition couple is a member of the group consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; said moiety being one of the recognition couple and said substance being or including the other. - -

-- 54. A method according to Claim 50, for assaying the presence of one or more target oligonucleotides with a specific target sequence in a sample, comprising:

(a) providing a substrate carrying one or more probe oligonucleotides each with a probing sequence complementary to one of said target sequences;

(b) contacting said substrate with the sample and providing conditions allowing formation of nucleation centers where a target oligonucleotide hybridizes to a probe oligonucleotide;

(c) contacting said substrate with a treatment composition comprising a gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(d) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating the presence of said substance at said site. - -

-- 55. A method according to Claim 54, wherein said step (b) comprises:

- (b1) treating said sample to bind a label to oligonucleotides in the sample;
- (b2) contacting said sample with said substrate; and
- (b3) contacting said substrate with a nucleation center-containing agent, capable of specific binding to said label. - -

-- 56. A method according to Claim 54, wherein said step (b) comprises:

- (b1) treating said sample so as to bind nucleation centers to oligonucleotides present therein; and
- (b2) contacting said samples with said substrate. - -

-- 57. A method according to Claim 50, wherein said nucleation center is a gold particle a cluster containing gold atoms, or a gold-containing complex or molecules. - -

-- 58. A method according to Claim 50, wherein said treatment composition is an aqueous solution. - -

- 59. A method according to Claim 58, wherein said gold-providing agent is $\text{Au}^{\text{I}}(\text{SCN})_2^+$ and said reagent is a quinone. - -
- 60. A method according to Claim 50, wherein said substrate is a specimen for viewing in an imaging or visualization technique. - -
- + - 61. A method according to Claim 60, wherein the detection technique is based on light. - -
- 62. A method according to Claim 60, wherein the { } detection technique is based on light absorbance and/or light transmission and/or light reflectance and/or light scattering. - -
- 63. A method according to Claim 60, wherein said detection is made by techniques selected from: transmission electron microscopy, scanning electron microscopy, atomic force microscopy. - -
- 64. A method according to Claim 60, for preparing the specimen for viewing in a microscope, comprising the following steps:
 - (a) providing conditions permitting formation of nucleation centers at selective sites of the specimen; and
 - (b) contacting said specimen with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is essentially not deposited on the specimen a nucleation center is present thereon, whereby gold atoms from said gold-providing agent are released and deposited onto said specimen at selective sites. - -
- 65. A method according to Claims 50, wherein said substrate contains separated fractions of a sample. - -
- 66. A method according to Claim 64, for identifying locations of a specific separation product on the substrate, comprising the steps of:
 - (a) providing conditions permitting formation of nucleation centers at sites on said substrate comprising said specific separation product;
 - (b) contacting said substrate to a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and

comprising a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only where a nucleation center is present thereon, whereby upon such contact, gold atoms from said gold-providing agent are released and deposited onto said nucleation center; and

(b) detecting presence of gold on said substrate, such gold signifying presence of said specific separation product on the substrate at a site where the gold is detected. - -

-- 67. A method for assaying presence of an analyte in a sample comprising:

(a) providing a substrate carrying capturing agents which bind said analyte;

(b) contacting said substrate with said sample, whereby said analyte binds to said capturing agents;

(c) providing conditions permitting formation of nucleation centers on sites of said substrate comprising said analyte;

(d) contacting said substrate with a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers; and

(e) detecting metallic gold deposits on said substrate, indicative of the presence of said analyte in said sample. - -

-- 68. A method according to Claim 66, wherein step (c) comprises:

(c1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster of metal atoms and a metal-containing complex; and

(c2) contacting said substrate with said agents.

-- 69. A method according to Claim 68, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a

nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said at least one moiety is the other of said binding couple. - -

-- 70. A method according to Claim 67, wherein step (b) comprises:

(b1) contacting said sample with nucleation centers, the nucleation centers being one or more of the group consisting of metal particles, cluster of metal atoms and metal-containing complexes or molecules, and providing conditions for coupling of said nucleation centers to the analyte if present in the sample, thus obtaining a modified sample containing modified analytes; and

(b2) contacting said substrate with said modified analytes bound to said nucleation centers. - -

-- 71. A method according to Claim 70, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said capturing agent is the other of said binding couple. - -

-- 72. A method according to Claim 67, wherein said capturing agents are carried on the substrate between electrodes such that gold deposited onto said nucleation centers in step (d) establishes an electric contact between the electrodes; and

detection of the gold deposits in step (e) is performed by measuring current-potential relationship between the electrodes. - -

-- 73. A method according to Claim 67, wherein said at least one metal particle, cluster of metal atoms or a metal-containing complex is a gold particle or cluster containing gold atoms. - -

- - 74. A method according to Claim 67, wherein said treatment composition is an aqueous solution. - -
- - 75. A method according to Claim 74, wherein said gold-providing agent is $\text{Au}^{\text{I}}(\text{SCN})_2^-$.
- - 76. A method according to Claim 74, wherein said reagent is a hydroquinone.
- - 77. A kit for use in the method according to Claim 42.
- - 78. A kit according to Claim 77, comprising a treatment composition which comprises a soluble gold-providing agent, being a gold-containing molecule or complex and comprises a reagent, the composition being kinetically stable such that upon contact with a substrate gold is deposited on a substrate essentially only at sites of the substrate containing nucleation centers. - -
- - 79. A kit according to Claim 77, further comprising nucleation center-forming agents, for forming nucleation centers at one or more sites on the substrate, said agents comprise each at least one {} member of a recognition group having a specific binding affinity to a substance present at said one or more sites coupled to at least one nucleation center moiety, being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex or molecule.
- - 80. A kit for use in a method according to Claim 67, for assaying an analyte in a sample, comprising:
 - (i) a substrate carrying capturing agents which bind said analyte;
 - (ii) agents for forming nucleation centers at portions of the substrate on which said analyte becomes immobilized;
 - (iii) a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers. - -
- - 81. A kit according to Claim 80, wherein said agent for forming nucleation centers comprises a nucleation center and substances needed in order to couple said nucleation center to said analyte. - -

-- 80. A kit according to Claim 80, further comprising nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center moiety being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex. --

-- 81. A kit according to Claim 80, wherein said substrate comprises two or more electrodes, and said capturing agents are carried within gaps between the electrodes. --

METHOD FOR GOLD DEPOSITION

FIELD OF THE INVENTION

The invention relates to chemical deposition of gold metal on substrates.

BACKGROUND OF THE INVENTION

There are various techniques and uses in which metal substances are deposited on substrates. In various such methods, the metal to be deposited is provided in a form of a soluble complex or molecule, e.g. in an aqueous solution. Under appropriate conditions the complex or molecule is reduced and deposits the metal onto the substrate.

At times, in order to ensure deposition of metal from solution onto a substrate, it is preferable to first provide nucleation centers on the substrate. Such nucleation centers may, for example, be a variety of metal complexes, clusters or small metal particles, deposited on or attached to the surface.

Examples of techniques involving metal deposition are a variety of image formation or latent image contrast-enhancement techniques for photography, optical microscopy or electron microscopy. Another example of laboratory techniques where metal complexes are deposited onto a substrate, is in imaging of separation products, e.g. proteins or nucleic acids, in a variety of chromatographic or electrophoretic techniques.

The metal most typically used in such techniques is silver. However, one typical drawback of silver deposition is that the deposition is not completely specific and some degree of spontaneous deposition of silver may occur also without an *a priori* deposition of nucleation centers. Thus, where silver deposition is used in image-enhancement or contrast-enhancement methods, there is typically a limited signal-to-noise ratio.

GENERAL DESCRIPTION OF THE INVENTION

The present invention has, as one of its objects, the provision of a method for depositing gold onto specific sites on a substrate. Another object of the invention is to provide a composition and kit for use in such a method.

5 In accordance with the present invention gold, initially contained in a soluble gold-providing agent, being a gold-containing molecule, cluster or complex, is deposited onto nucleation centers. The term "*nucleation centers*" as used herein refers to any salt, complex, cluster or particle having catalytic properties of transforming gold from a gold-containing molecule or complex into
10 gold metal. Particles with a metal surface are, for example, colloid metal particles, a variety of metal complexes, clusters containing metal atoms, etc.

In accordance with the invention there is provided a method for depositing gold at one or more sites on a substrate, comprising:

- (a) binding, depositing or forming nucleation centers at said one or more
15 sites;
- (b) contacting said one or more sites with a treatment composition comprising said gold-providing agent and a reagent, the composition being kinetically stable such that gold metal is essentially not deposited on the substrate unless a nucleation center is present on the substrate and in the presence of a
20 nucleation center at said one or more sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said one or more sites.

As will be appreciated, once gold is deposited on the substrate, it serves as a nucleation center for further gold deposition. By appropriately controlling the
25 incubation time as well as other conditions affecting the gold deposition (some of which will be outlined bellow), the amount of the deposited gold at a deposition site can be controlled. Furthermore, although the initial gold deposition may be at discrete sites on the substrate, by allowing the gold deposits to grow, following further deposition, discrete sites may combine to form one larger gold deposit
30 encompassing the so combined sites.

The term "*essentially not deposited*" means to denote that there is either no gold separation from the solution and the gold atoms remain within the soluble molecule or complex, or that gold metal deposition on a substrate is negligible and thus not or only hardly observable and measurable, yielding a high signal-to-noise ratio.

In accordance with one embodiment, the nucleation centers are formed by chemical or physical deposition of nucleation center forming agent onto the one or more sites. By another embodiment, such nucleation centers may be formed chemically at said one or more sites by a redox reaction in which metal ions, in particular silver ions, are oxidized into metallic silver. Such chemical formation of nucleation centers is known *per se* (see for example WO 99/04402 and PCT/IL99/00232).

In accordance with yet another embodiment, the nucleation centers are formed by binding of nucleation center-forming agents at said one or more sites. Such agents comprise at least one binding moiety with a binding affinity (which may be specific or non-specific) to said one or more sites, which is bound to at least one nucleation center moiety. The nucleation center moiety is a species that can act as a catalytic agent for gold metal deposition, e.g. a salt, metal particle, cluster of metal atoms or a metal-containing complex. The binding moiety may, for example, be one member of a recognition group, where another member of the recognition group forms or is included in said one or more sites. The recognition group, typically a recognition couple, consists of two or more substances with a binding affinity one to the other. The recognition group may be, but is not limited to, any one of the following couples: an antigen and an antibody or an antibody derivative with a complementary antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or synthetic binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain.

The nucleation center moiety is preferably a gold particle or a cluster containing gold atoms, e.g. Au₁₁ or Au₅₅ or Au₁₄₇ (gold clusters that contain 11, 55

or 147 gold atoms, respectively). The treatment composition is typically an aqueous solution. Said gold-providing agent is preferably a gold complex such as $\text{Au}^{\text{I}}(\text{SCN})_2^-$ usually in the form of an alkali metal (e.g. Na^+ or K^+) or ammonium salt.

5 The reagent in the treatment composition may be a reducing agent. Where said gold-providing agent is $\text{Au}^{\text{I}}(\text{SCN})_2^-$ the reagent is preferably a quinone, e.g. hydroquinone (HQ) or naphthohydroquinone (NHQ). However, any other agent which can reduce a gold-containing molecule or complex to obtain gold metal, but because of kinetic limitations (e.g. high activation energy and low pre-exponential
10 factor) will do so in a negligible rate in the absence of a nucleation center, yet, in a meaningful rate (namely in a rate that yields observable results in a not too long period of time) when a nucleation center is present, may also be used.

A treatment composition comprising $\text{Au}^{\text{I}}(\text{SCN})_2^-$ and HQ in an acidic medium is stable, meaning that gold remains as soluble species in solution and
15 neither precipitates as solid metal nor is deposited on solid substrates other than nucleation centers. When the pH of such a treatment composition is below about 6, the treatment composition is particularly stable. Upon contact with a nucleation center gold metal is deposited on sites of the substrate comprising nucleation centers.

20 The rate of deposition of the gold metal may be controlled, for example, by controlling the pH of the treatment composition: increase in pH will increase the rate of gold deposition and a decrease in pH will decrease the deposition rate. Furthermore, the rate of deposition may also be controlled by a variety of other means, for example: by controlling the flow parameters of the treatment
25 composition (rapid flow prevents poisoning by by-products, such as CN^- , which can reduce the rate of gold deposition); by adding surface active agents which can either increase or decrease the rate of deposition (for example polyamines, poly acids or polyalcohols and other surface active agents may have the effect of altering the rate of gold deposition). In addition, by the use of various additives, e.g. those
30 noted above, the roughness of the surface of the deposited gold may be changed

(such a change may result in a change of color and electrical properties of the deposited gold).

The method of the invention may be used for assaying the presence or concentration of a specific substance at sites on a substrate. Such a method
5 comprises the following steps:

(a) providing conditions allowing formation of nucleation centers on sites containing said substance;

(b) contacting said substrate with a treatment composition comprising said gold-providing agent and a reagent, the composition being kinetically stable
10 such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation centers to form gold metal at said sites; and

15 (c) detecting gold deposits on said substrate, a gold deposit at a site on the substrate indicating presence of said substance at said site.

The assay method may be applicable, for example, in a variety of assay techniques, e.g. in techniques involving visualization of specimens in microscopy (which may be an optical or an electron microscope) or any SPM (_____)
20 technique, or in identifying specific separation products on a substrate (for example, a separation product of electrophoresis or chromatography contained in a gel or on a solid substrate such as DNA-chip). In such an assay, the nucleation centers may be formed by the use of said nucleation center-forming agents where the moiety with the specific binding affinity is one member of a recognition group,
25 which may be any of those mentioned above, and the agent to be assayed is another member of the group.

In accordance with another embodiment, the gold deposition method of the invention is used in an assay intended to detect the presence of an analyte in a sample. In particular, the present invention is applicable to such a method where a
30 capturing agent held on a substrate is used to detect the presence of an analyte in a

sample that binds specifically to the capturing agent. Such a capturing agent may be a member of a recognition group, while the analyte is another member of the group. A specific example is the case of assaying for the presence of a target oligonucleotide in a sample, with the capturing agent being an oligonucleotide with a sequence complementary thereto. A nucleation center forming moiety may be bound to analytes presence in the sample, e.g. to the oligonucleotides in the sample, and then after allowing the sample to react with the substrate, and performing a gold deposition reaction as described above, formation of gold deposits will indicate the presence of the analyte in the sample.

10 A method of the invention of preparing a specimen for imaging in a microscope, comprises the following steps:

(a) providing conditions permitting formation of nucleation centers at selective sites of the specimen; and

(b) contacting said specimen with a treatment composition comprising said gold-providing agent and a reagent, the composition being kinetically stable such that gold is essentially not deposited on the specimen unless a nucleation center is present thereon, whereby gold atoms from said gold-providing agent are released and deposited onto said specimen at said selective sites.

A method of the invention for detecting the presence of an analyte in a sample, comprises the following steps:

(a) treating the sample to bind nucleation center forming moieties to the analyte, if present in the sample;

(b) reacting the sample with a substrate, holding capturing agents that specifically bind said analyte, and removing unbound sample portions;

25 (c) contacting said substrate with a treatment composition comprising said gold-providing agent and a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing

agent and deposited onto said nucleation centers to form gold metal at said sites;
and

(d) detecting gold deposits on said substrate, a gold deposit indicating presence of the analyte in the sample.

5 A specific application of this method is in the oligonucleotide chips. Oligonucleotide chips have different capturing oligonucleotides deposited in different sites on the chip. Applying the above method may allow to simultaneously assay for a large number of different oligonucleotides in a sample.

In a so prepared specimen, said selective sites thus contain gold deposits
10 which permit their visualization. Said selective sites may typically be sites containing a specific substance and the nucleation centers are then formed at said sites by nucleation center forming agents with a moiety having specific binding affinity to said specific substance. In this manner, for example, specific cells, organelles, regions rich with a specific substance, etc. can be clearly visualized.

15 A method according to the invention for identifying locations of a specific separation product on a substrate, comprises the following steps:

(a) providing conditions permitting formation of nucleation centers at sites on said substrate comprising said specific separation product;

(b) contacting said substrate with a treatment composition comprising
20 said gold-providing agent and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only where a nucleation center is present thereon, whereby upon such contact, gold atoms from said gold-providing agent are released and deposited onto said nucleation center to form gold metal; and

25 (c) detecting presence of gold on said substrate, such gold signifying presence of said specific separation product on the substrate at a site where the gold is detected.

In a separation technique where the sample is separated into various fractions based on different movement rates of different substances through a
30 medium (typically a gel or a solid) under applied conditions, for example

electrophoresis, thin layer chromatography (TLC), etc., the nucleation centers-forming agents may be admixed to the sample prior to separation. This is usually preferred. For one such prior addition of the nucleation center-forming agent may be more economical, as less material may be required. In addition, where
5 such agents are applied to the substrate after the separation, they have to diffuse through the medium in order to reach the separated substance located within the medium; such diffusion may be a limiting factor in proper gold-staining of separated fractions. However as will readily be appreciated, it is possible at times to add the nucleation center-forming agent also after separation.

10 The present invention also provides a method for assaying presence of an analyte in a sample. Such a method comprises the following steps:

- (a) providing a substrate carrying capturing agents which bind said analyte;
- (b) contacting said substrate with said sample, whereby said analyte
15 binds to said capturing agents;
- (c) providing conditions permitting formation of nucleation centers on sites of said substrate comprising said analyte;
- (d) contacting said substrate with a treatment composition comprising said gold-providing agent and a reagent, the composition being kinetically stable
20 such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers; and
- (e) detecting metallic gold deposits on said substrate, indicative of the presence of said analyte in said sample.

The detection in step (e) of the method for assaying presence of an analyte in
25 a sample, may be carried out by testing appearance of gold deposited on the substrate (gold deposited, depending on size and roughness, may have a color ranging from yellow to orange to red).

In accordance with one embodiment of the invention, the detection in step (e) is based on the electric conductivity of the deposited gold. In this
30 embodiment the capturing agents may be carried on the substrate between

electrodes or may be carried on one or more electrodes such that the gold deposited onto the nucleation center in step (d) establishes an electric contact between electrodes. The detection of the gold deposits in step (e) is thus carried out by determining existence of electrical contact between the electrodes, e.g. by measuring the current-potential relationship between the electrodes. The magnitude of the current-potential relationship may be used as a gauge for the agent's concentration in the sample. Alternatively, determining the number of connections in a large array of similar electrode couples with identical capturing agents carried between or on them, may also provide a quantitative measure of the analyte concentration.

The assayed analyte may be a member of any of the recognition couples mentioned above, with the other member of the couple being then included as a moiety in the capturing agent. The formation of the nucleation centers in step (c) may be the provision of agents having a binding moiety with specific binding affinity to the analyte captured on the substrate by the capturing agent, bound to a nucleation-forming moiety, which may be a metal particle, a cluster containing metal atoms or a metal-containing complex, or any other species having the catalytic properties of transforming gold-containing molecular species into gold metal. For example, where the analyte is one member of a binding couple, the binding moiety may be another member (e.g. where the analyte is an antigen, the nucleation center-forming agent may comprise an antibody, which may be the same or different as an antibody used as the capturing agent). The present invention further provides a kit for use in the above method. Such a kit typically comprises a treatment composition. In addition, such a kit may also comprise a reagent for forming nucleation centers at specific sites on a substrate, e.g. such having a specific binding moiety being a member of any of the above binding couples. In addition, a kit for use in the above assay method may also comprise, according to some embodiments, a reagent system for tagging an analyte in a sample with nucleation center-forming moiety. Furthermore, in accordance with some

embodiments of the invention, the kit may comprise a substrate comprising electrodes with capturing agents disposed between and/or on the electrodes.

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting
5 example only, with occasional reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows reaction schemes of the manner of preparation of a treatment composition in accordance with an embodiment of the invention (Scheme (a) and Scheme (b)) and of gold deposition onto a nucleation center (Scheme (c)).

10 Fig. 2 is a schematic representation of an assay of an analyte in a sample in accordance with an embodiment of the invention.

Fig. 3 is a schematic representation of an assay in accordance with another embodiment of the invention.

15 Fig. 4 is a schematic representation of an assay in accordance with a further embodiment of the invention.

Fig. 5 is a schematic illustration of the manner of enhancing specific bands on a solid or a gel substrate, separated by chromatography or by electrophoresis.

Fig. 6 is a schematic illustration of a method in accordance with an embodiment of the invention for imaging specific parts of a microscope specimen.

20 Fig. 7 shows the application of the system in the detection of analytes, oligonucleotides with a specific sequence in this specific example, in a sample.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Preparation of a treatment composition and general gold deposition scheme

25

A treatment composition in accordance with the preferred embodiment of the invention comprises a gold-containing complex- $\text{Au}^{\text{I}}\text{SCN}^-$. In order to prepare such a treatment composition at a first stage a solution comprising $\text{KAu}^{\text{III}}\text{Cl}_4$ and KSCN is prepared, typically by mixing two stock solutions, one comprising the

gold containing complex and the other the KSCN. For example, such a solution may be formed by mixing equal volumes of a first solution comprising about 0.5 M KSCN and a second solution comprising about 0.05 M $\text{KAu}^{\text{III}}\text{Cl}_4$. Such mixing results in the formation of the complex $\text{KAu}^{\text{III}}(\text{SCN})_4$ (See Scheme (a) in Fig. 1).

5 This complex precipitates but spontaneously forms the complex $\text{KAu}^{\text{I}}(\text{SCN})_2$. Typically, after the $\text{KAu}^{\text{III}}(\text{SCN})_4$ is precipitated, it is washed and resuspended in water or buffer and allowed to undergo the spontaneous reaction (SPONT.) noted above (illustrated in Scheme (b) of Fig. 1).

The $\text{KAu}^{\text{I}}(\text{SCN})_2$ solution is then mixed with a reducing agent, e.g. with
10 about 0.055 M hydroquinone (HQ). The reduction of the gold-containing complex by the hydroquinone is then thermodynamically favorable, but owing to the large activation energy and low pre-exponential factor, the kinetics is very slow. At a pH of less than about 6, the spontaneous reduction is practically nil. Thus, the solution is stable and gold metal is not deposited spontaneously.

15 However, as shown in Fig. 1, scheme (c), upon exposure to nucleation centers (NC), acting as catalysts, which may for example be gold colloids, other metal colloids, clusters of metal atoms, metal-containing complexes, metal ions, etc., gold metal (Au^0) is being deposited on the surface of the nucleation center associated with the formation of benzoquinone (BQ).

20 The pH level controls the rate of gold deposition: acidification of the solution, namely lowering pH, slows the metal deposition, while addition of a base, namely increasing pH, accelerates the rate of metal deposition.

It should be noted that under basic pH, some spontaneous deposition of gold may take place even without nucleation centers.

25 Various additives which may either be added initially to the treatment solution, or added during the deposition process, may influence the rate of the gold deposition process and the pattern of the gold deposit (size of deposits, surface roughness, color, etc.). For example, addition of halogen ions to the treatment composition results in surface poisoning of the growing metal centers.

30 This will reduce the rate, and eventually stop the gold deposition process. Use of

such additives thus allows a control of the deposited gold crystallinity, grain size, the rate of the gold deposition, the roughness of the surface of the deposited gold, the electrical properties, etc. The gold deposition process may obviously also be controlled by the change of various other parameters such as temperature, oxygen
5 content, light flux, solution-agitation rate, etc.

The gold deposition process of the invention can be used for a variety of diverse applications, some examples of which will be illustrated below.

Detection of analytes in a sample

10 An assay in accordance with one embodiment of the invention is illustrated in Fig. 2. Provided by this embodiment is a device 20 having a non conductive substrate 22 carrying electrically conductive electrodes 23. In the gap between electrodes the substrate 22 carries capturing agents 24 which have a specific binding affinity to analyte 26 to be assayed.

15 Sample S, which comprises analyte 26, is pretreated by adding nucleation centers 28 and treating it in a manner so that nucleation centers will become coupled to the analyte in a covalent or non covalent interaction, to yield modified analyte 30. In the case of an oligonucleotide analyte, such coupling may, for example, be performed by heating the sample in the presence of a
20 cis-platinum-biotin and then adding an appropriately derivatized cluster, e.g. $\text{Au}_{55}(\text{Ph}_3\text{P})_{12}\text{Cl}_6$ -streptavidin cluster. The cis-platinum-biotin binds to the oligonucleotides, then $\text{Au}_{55}(\text{Ph}_3\text{P})_{12}\text{Cl}_6$ - streptavidin binds to the biotin moieties on said nucleotides, thus binding nucleation moieties to the analyte. In the case of a non-nucleotide analyte, coupling may be achieved, for example by binding
25 amino-derivatized cluster or colloid to carboxy moieties of the analyte; coupling carboxy-derivatized clusters or colloids to amino-moieties of the analyte; coupling maleimido-derivatized cluster or colloids to thiol moieties of the analyte; etc. As will be appreciated, other substances in a sample may also become tagged with the nucleation center in this process. However, these will not

bind to capturing agents 24 and the nucleation centers coupled thereto will thus not remain on the substrate after treatment. (See description below).

Upon contact with device 20, modified analyte 30 binds to the capturing agents to yield nucleation center-forming complexes 32 which are immobilized
5 on substrate 22. Washing the substrate removes unbound substances (NB).

Device 20 may then be contacted with the treatment solution 34 which comprises a gold containing complex such as $\text{KAu}^{\text{I}}(\text{SCN})_2$ and a reducing agent such as hydroquinone (HQ) and consequently upon contact with the nucleation centers 28, gold is deposited on the nucleation centers and forms a continuous
10 gold mass 36 extending between electrodes 23. In order to avoid that electrodes 23 will act as nucleation centers, they may either be made of a conductive material lacking catalytic properties, e.g. silicon passivated with silica, with long alkyl chains, or passivated by coating with an inert material, or, where the electrodes are made of a metal, they may be pretreated to render them
15 inert as gold-deposition catalysts, e.g. by incubation with a solution comprising a long chain alkane thiol, such as 1-octadecyl-thiol.

By measuring current voltage relationships using a measurement device 40, the existence of an electric contact between the electrodes 23 can be determined, the presence of which indicates the presence of the analyte in the
20 assayed sample.

As can generally be appreciated by the artisan, the binding of the capturing agents to the substrate may be achieved in a number of ways. For example, oxide surfaces may be derivatized with a silicon reagent such as $(\text{CH}_3\text{CH}_2\text{O})_3\text{Si-R-X}$, wherein R may be an alkyl, aryl or other spacer and X may be an active moiety
25 for subsequent binding to a moiety Y in the capturing agent. The nature of X and Y depends on the couple and may be selected from couples consisting of acid and amine, hydroxy and acid, cycloaddition reactions, radical reactions, nucleophilic substitutions, non-covalent interactions, etc., as is well known and clear to the artisan. Where the substance is made of a polymeric material, binding, by any of

the above type of reactions, or others, may also be achieved between the capturing agent and a side group of the polymeric material.

Device 20 may comprise two or more electrodes, typically a plurality of electrodes arranged in an array. The array may assume different geometries. The same capturing agents may be carried between different pairs of electrodes, and/or different capturing agents may be deposited between different electrodes. A device of the latter kind may be used in the multiplexing assay for simultaneous determination of a number of analytes. In addition, where the same capturing agents are carried between different pairs of electrodes, this may allow to perform a quantitative assay of concentration of the analyte: for example, the concentration may be assessed based on the number of electric contacts formed between different pairs of electrodes - a large number of electric contacts signifying a large concentration and a small number corresponding to a small concentration.

The electrode array may also be part of a more complex electronic setup containing different components, e.g. diodes, transistors, conductors, capacitors, etc.

Another embodiment of an assay in accordance with the invention can be seen in Fig. 3. The main difference in the case of the embodiment of Fig. 3 from that described in Fig. 2, is that rather than treating sample S to bind nucleation centers 28 to analytes 26 to obtain a modified analyte 30, in the case of the embodiment of Fig. 3, a nucleation center-forming agent 150 is provided. Other components of this assay are very similar to those of Fig. 2 and thus the same reference numeral shifted by one hundred (namely with a "1" prefix) to those used in Fig. 2 were employed in Fig. 3 to indicate like components.

After contact of a device 120 with a sample, analyte 126 binds to a capturing agent 124 and then after washing free (non bound (NB)) analyte molecules and other substances in the sample, the device is contacted with a nucleation center-forming agent 150 which comprises a moiety 152 with a specific binding affinity to analyte 126 which is now bound to capturing

agents 124, coupled to a nucleation center 154. Thus, an immobilized nucleation center 132 forms on the substrate in the gap between electrodes 123. Then, after removal of non bound agents 150 (NB) and adding a treatment composition 134 the gold mass 136 forms which provides electric contact between electrodes 123, which may then be determined similarly as shown in Fig. 2.

A further embodiment of an assay in accordance with the invention is seen Fig. 4. In Fig. 4, like elements to those of Figs. 2 and 3 have been marked with the same reference numeral to that used in Fig. 2 shifted by one hundred (namely with a "2" prefix). Sample S containing analyte 226 is reacted with biotin 227 to yield a treated sample S' with analyte-biotin complexes 231. These are then contacted with substrate 222 which contain capturing agents 224 fitted between electrodes 223, to yield modified capturing complexes 233 on substrate 222.

Gold colloid particles 240 are reacted with an avidin or streptavidin 242 to yield nucleation center forming agents 243. Contacting agent 243 with modified substrate 222 then yields immobilized nucleation centers 235. The subject can then be contacted with a treatment solution 234 similarly as in Figs. 2 and 3 resulting in gold deposition to form a continuous gold mass 236 between electrodes 223.

As will no doubt be appreciated by the artisan, various modifications of the embodiments shown above are possible.

Visualization of separation products

The method of the invention can be used for visualizing separation product obtained in a number of separation techniques such as gel electrophoresis, gel permeation techniques, size exclusion chromatography, affinity chromatography and others. This is illustrated schematically in Fig. 5.

Substrate 200, which may be a gel or a solid substrate containing different lanes 202, each consisting of a plurality of bands 204, each one being a specific fraction of a sample separated in each lane. Such a substrate 200 may be obtained by any of the techniques noted above.

In order to visualize the existence of a specific substance and locate its band, a nucleation center-forming agent 206 which has a moiety 208 which specifically binds to the substance in a nucleation center 210 is contacted with substrate 200 and after access, agents 206 are washed away, the substrate is
5 contacted with a treatment solution 212 and consequently gold deposits 214 will form on bands containing that substance (the other bands are not visible and are included only for illustration purposes). Preferably, however, nucleation center-forming agents 206 may be added to the substrate prior to the beginning of the separation process since diffusion of subsequently-added nucleation
10 center-forming agents may be a limiting factor to achieve binding of these agents to the separated substance.

Depending on the exact deposition conditions, which will *inter alia* determine the roughness of the surfaces of the deposited gold mass, the color of the bands may vary between yellow to black. Using various visualization
15 techniques, based on measurement of light absorbance or transmission through the bands, a quantitative measurement of a separate substance may be obtained.

In the alternative, rather than optical techniques, the gold deposits may also be detected by various electric detection techniques. This may be achieved for example by providing two planar arrays of electrodes sandwiching the
20 separation matrix which will permit an automatic electric detection.

Contrast enhancing in imaging techniques

There are many imaging techniques which request contrasting agents in order to view certain components. Such is the case in optical microscopy, electron microscopy (transmission electron microscopy, scanning electron
25 microscopy) as well as in other imaging techniques, such as atomic force microscopy. Hitherto, such imaging techniques involved silver metal deposition or carbon deposition and some others.

In the case of biological specimens, contrast enhancers are required to
30 allow visualization of specific tissues, cells, organelles, etc.

In accordance with the invention, as illustrated in Fig. 6, a biological specimen 300, containing a cell 302 with a non visible organelle 304, is treated in accordance with the invention by successive exposure to nucleation center-forming agent 306 which has a binding moiety 308 specific to substance included in organelle 304 and a nucleation center 310 and after treatment with a treatment composition 312, organelle 304 is visible as a dark or opaque deposit 314.

Imaging of oligonucleotide hybridization

The assaying of the presence and concentration of specific-sequence oligonucleotides is nowadays typically being performed by the use of oligonucleotide probes of a complementary sequence attached to known locations on a substrate. Specific and widely used examples of such systems are the so-called DNA-chips. Such chips are surfaces bearing a plurality of oligonucleotide-probe sequences in known and discrete locations on a surface. A sample that may or may not contain the target sequences that complement the probe sequences on chip is treated so that all DNA fragments are labeled (usually by fluorescent labels). Contacting the sample with the surface of the chip under appropriate conditions, the target oligonucleotides attach to the surface in locations bearing the complementary sequence. Detection of hybridization products is usually done by following the fluorescence of labeled duplexes. The location of hybridization product indicates the sequence of the probe and fluorescence intensity indicates their surface concentration.

Reference is now being made to Fig. 7 showing the application of the gold deposition method of the invention in visualization of hybridization products on a DNA-chip using different possible visualization techniques such as optical techniques (absorption, reflectance) and non optical visualization techniques (AMF, STM etc.). As no doubt will be appreciated, this is but an example to the use of the method of the invention for visualizing binding of targets to probes in

general, e.g. binding of c-DNA strands to complementary strands, a binding of an antibody to an antigen and other types of recognition couples.

A DNA-chip substrate 400 carries a plurality of DNA probe sites, three 402, 404 and 406 being exemplified, each one having a different nucleotide sequence. A sample S that may contain the target oligonucleotides 410 is treated in a way that all DNA species in solution become labeled by label 412, thus obtaining modified sample S' that may contain modified analyte (if analyte was present in S). Contacting modified sample S' with DNA-chip substrate 400 under appropriate conditions allows selective hybridization of DNA probes on chip to target oligonucleotides if present in modified sample S'. After washing the chip, nucleation centers-forming agents 430 are introduced to the system under condition allowing their binding to labels 412 on labeled oligonucleotides if present at the different sites on chip. After washing, nucleation centers are present only at locations where hybridization between probes on chip and target from solution occurred. Visualization of hybridization is achieved by applying the gold deposition method of the invention, thus growing gold grains from the nucleation centers on-chip and detecting the gold 440 grains using different techniques such as optical techniques and SPM techniques (AFM, STM etc.).

In another embodiment, the label serves itself as a nucleation center. In such case, the step of attaching the nucleation center to the label is skipped.

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25 3. A method according to Claim 1, wherein the one member of the recognition group is coupled to at least one nucleation center being one or more of the group consisting of gold particle, cluster containing gold atoms and gold-containing complexes and molecules.

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4. A method according to Claim 1, wherein the one member of the recognition group is coupled to at least one nucleation center being one or more of the group consisting of cluster containing gold atoms and gold-containing complexes and molecules.
- 5 5. A method according to Claim 1, wherein said {} recognition group is a member of the group consisting of: an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin
10 and avidin or streptavidin; cellulose or chitin and cellulose binding domain.
6. A method according to any one of Claims 1 to 5, wherein said treatment composition is an aqueous solution.
7. A method according to Claim 6, wherein said gold-providing agent is $\text{Au}^+(\text{SCN})_2^-$.
- 15 8. A method according to Claim 7, wherein said reagent is hydroquinone or naphthohydroquinone.
9. A method for assaying presence of a specific substance at sites on a substrate, comprising:
 - (a) providing conditions allowing formation of nucleation
20 centers on sites containing said substance;
 - (b) contacting said substrate with a treatment composition comprising a soluble gold providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is
25 essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

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(c) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating presence of said substance at said site.

10. A method according to Claim 9, wherein step (a) comprises:

5 (a1) providing nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said substance, bound to at least one nucleation center being one or more of the group consisting of metal particle, cluster containing metal atoms and a metal-containing complex; and

10 (a2) contacting said substrate with said agents.

11. A method according to Claim 10, wherein said moiety is included in one member of a recognition couple consisting of two molecules or complexes which specifically bind to one another, the other member included in or constituting said substance.

15 12. A method according to Claim 11, wherein said recognition couple is a member of the group consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other
20 specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; said moiety being one of the recognition couple and said substance being or including the other.

13. A method according to any one of Claims 9-12, for assaying the presence of one or more target oligonucleotides with a specific target
25 sequence in a sample, comprising:

(a) providing a substrate carrying one or more probe oligonucleotides each with a probing sequence complementary to one of said target sequences;

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(b) contacting said substrate with the sample and providing conditions allowing formation of nucleation centers where a target oligonucleotide hybridizes to a probe oligonucleotide;

(c) contacting said substrate with a treatment composition
5 comprising a gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that upon such exposure gold metal is essentially not deposited on the substrate unless a nucleation center is present thereon, and in the presence of a nucleation center at said
10 sites, gold atoms are released from said gold-providing agent and deposited onto said nucleation center to form gold metal deposits at said sites; and

(d) detecting metallic gold deposits on said substrate, a gold deposit at a site on the substrate indicating the presence of said
15 substance at said site.

14. A method according to Claim 13, wherein said step (b) comprises:

(b1) treating said sample to bind a label to oligonucleotides in the sample;
(b2) contacting said sample with said substrate; and
(b3) contacting said substrate with a nucleation center-containing agent,
20 capable of specific binding to said label.

15. A method according to Claim 13, wherein said step (b) comprises:

(b1) treating said sample so as to bind nucleation centers to oligonucleotides present therein; and
(b2) contacting said samples with said substrate.

25 16. A method according to any one of Claims 9 to 15, wherein said nucleation center is a gold particle a cluster containing gold atoms, or a gold-containing complex or molecules.

17. A method according to any one of Claims 9 to 16, wherein said treatment composition is an aqueous solution.

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18. A method according to Claim 17, wherein said gold-providing agent is $\text{Au}^{\text{I}}(\text{SCN})_2^-$ and said reagent is a quinone.
19. A method according to any one of Claims 9 to 18, wherein said substrate is a specimen for viewing in an imaging or visualization technique.
- 5 20. A method according to Claim 19, wherein the detection technique is based on light.
21. A method according to Claim 19, wherein the {} detection technique is based on light absorbance and/or light transmission and/or light reflectance and/or light scattering.
- 10 22. A method according to Claim 19, wherein said detection is made by techniques selected from: transmission electron microscopy, scanning electron microscopy, atomic force microscopy.
23. A method according to Claim 19, for preparing the specimen for viewing in a microscope, comprising the following steps:
- 15 (a) providing conditions permitting formation of nucleation centers at selective sites of the specimen; and
- (b) contacting said specimen with a treatment composition comprising a soluble gold-providing agent, being a gold-containing molecule or complex and comprising a reagent, the composition being kinetically stable such that gold is
- 20 essentially not deposited on the specimen a nucleation center is present thereon, whereby gold atoms from said gold-providing agent are released and deposited onto said specimen at selective sites.
24. A method according to any one of Claims 9 to 18, wherein said substrate contains separated fractions of a sample.
- 25 25. A method according to Claim 23 for identifying locations of a specific separation product on the substrate, comprising the steps of:
- (a) providing conditions permitting formation of nucleation centers at sites on said substrate comprising said specific separation product;
- (b) contacting said substrate to a treatment composition comprising a
- 30 soluble gold-providing agent, being a gold-containing molecule or complex and

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comprising a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only where a nucleation center is present thereon, whereby upon such contact, gold atoms from said gold-providing agent are released and deposited onto said nucleation center; and

- 5 (b) detecting presence of gold on said substrate, such gold signifying presence of said specific separation product on the substrate at a site where the gold is detected.

25. A method for assaying presence of an analyte in a sample comprising:

- 10 (a) providing a substrate carrying capturing agents which bind said analyte;

(b) contacting said substrate with said sample, whereby said analyte binds to said capturing agents;

- (c) providing conditions permitting formation of nucleation centers on
15 sites of said substrate comprising said analyte;

(d) contacting said substrate with a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers; and

- 20 (e) detecting metallic gold deposits on said substrate, indicative of the presence of said analyte in said sample.

26. A method according to Claim 25, wherein step (c) comprises:

- (c1) providing nucleation center-forming agents which comprise each at
25 at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center being one or more of the group consisting of metal particle, cluster of metal atoms and a metal-containing complex; and

(c2) contacting said substrate with said agents.

27. A method according to Claim 26, wherein said analyte is one of a binding couple consisting of an antigen and an antibody or an antibody derivative
30 with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a

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nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said at least one moiety is the other of said binding couple.

5 28. A method according to Claim 25 wherein step (b) comprises:

(b1) contacting said sample with nucleation centers, the nucleation centers being one or more of the group consisting of metal particles, cluster of metal atoms and metal-containing complexes or molecules, and providing conditions for coupling of said nucleation centers to the analyte if present in
10 the sample, thus obtaining a modified sample containing modified analytes; and

(b2) contacting said substrate with said modified analytes bound to said nucleation centers.

29. A method according to Claim 28, wherein said analyte is one of a binding
15 couple consisting of an antigen and an antibody or an antibody derivative with an antigen-binding domain; sugar and a lectin; a receptor and a ligand; a nucleotide sequence and a complementary nucleotide sequence; a nucleotide sequence and its binding protein or other specific binding agent; biotin and avidin or streptavidin; cellulose or chitin and cellulose binding domain; and said capturing agent is the
20 other of said binding couple.

30. A method according to any one of Claims 25 to 29, wherein said capturing agents are carried on the substrate between electrodes such that gold deposited onto said nucleation centers in step (d) establishes an electric contact between the electrodes; and

25 detection of the gold deposits in step (e) is performed by measuring current-potential relationship between the electrodes.

30. A method according to any one of Claims 25 to 30, wherein said at least one metal particle, cluster of metal atoms or a metal-containing complex is a gold particle or cluster containing gold atoms.

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32. A method according to any one of Claims 25 to 31, wherein said treatment composition is an aqueous solution.

33. A method according to Claim 32, wherein said gold-providing agent is $\text{Au}^{\text{I}}(\text{SCN})_2^-$.

34 A method according to Claim 32, wherein said reagent is a hydroquinone.

35. A kit for use in the method according to any one of Claims 1 to 34

36. A kit according to Claim 35, comprising a treatment composition which comprises a soluble gold-providing agent, being a gold-containing molecule or complex and comprises a reagent, the composition being kinetically stable such that upon contact with a substrate gold is deposited on a substrate essentially only at sites of the substrate containing nucleation centers.

37. A kit according to Claim 35 or 36, further comprising nucleation center-forming agents, for forming nucleation centers at one or more sites on the substrate, said agents comprise each at least one {} member of a recognition group having a specific binding affinity to a substance present at said one or more sites coupled to at least one nucleation center moiety, being one or more of the group consisting of metal particle, cluster of metal atoms and a metal containing complex or molecule.

38. A kit for use in a method according to any one of Claims 25 to 34, for assaying an analyte in a sample, comprising:

(i) a substrate carrying capturing agents which bind said analyte;
(ii) agents for forming nucleation centers at portions of the substrate on which said analyte becomes immobilized;

(iii) a treatment composition comprising a soluble gold-containing molecule or complex and a reagent, the composition being kinetically stable such that gold is deposited on the substrate essentially only at sites thereof containing the nucleation centers .

39. A kit according to Claim 38, wherein said agent for forming nucleation centers comprises a nucleation center and substances needed in order to couple said nucleation center to said analyte.

ART 31 PART

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40. A kit according to Claim 38, further comprising nucleation center-forming agents which comprise each at least one moiety, having specific binding affinity to said analyte, coupled to at least one nucleation center moiety being one or more of the group consisting of metal particle, cluster of metal atoms and a metal
s containing complex.

41. A kit according to any one of Claims 38 to 40, wherein said substrate comprises two or more electrodes, and said capturing agents are carried within gaps between the electrodes.

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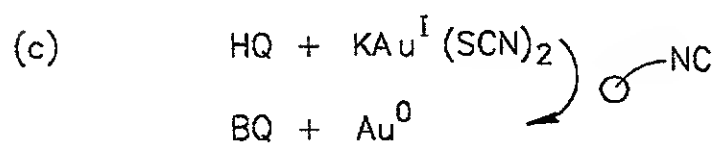


FIG.1

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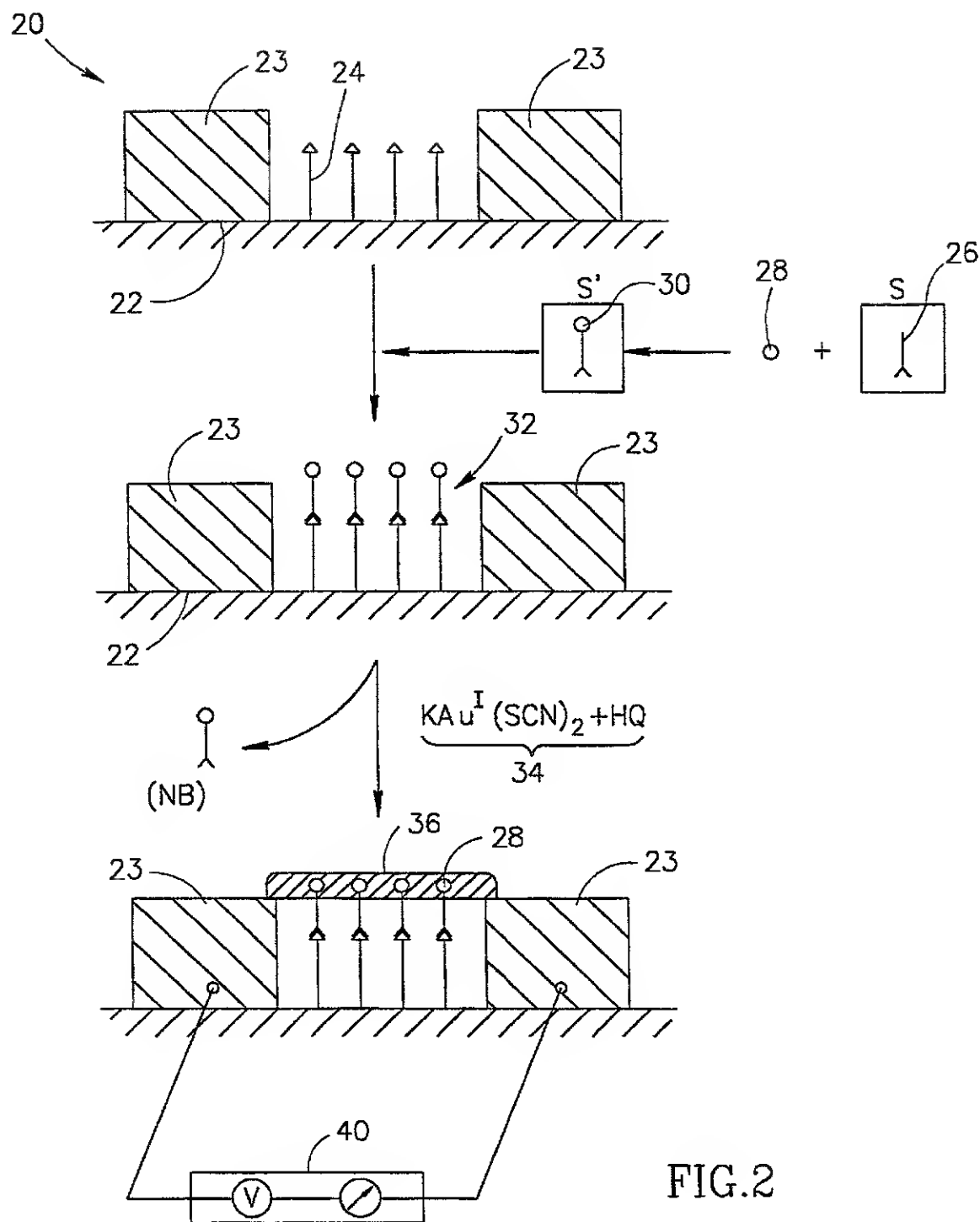


FIG.2

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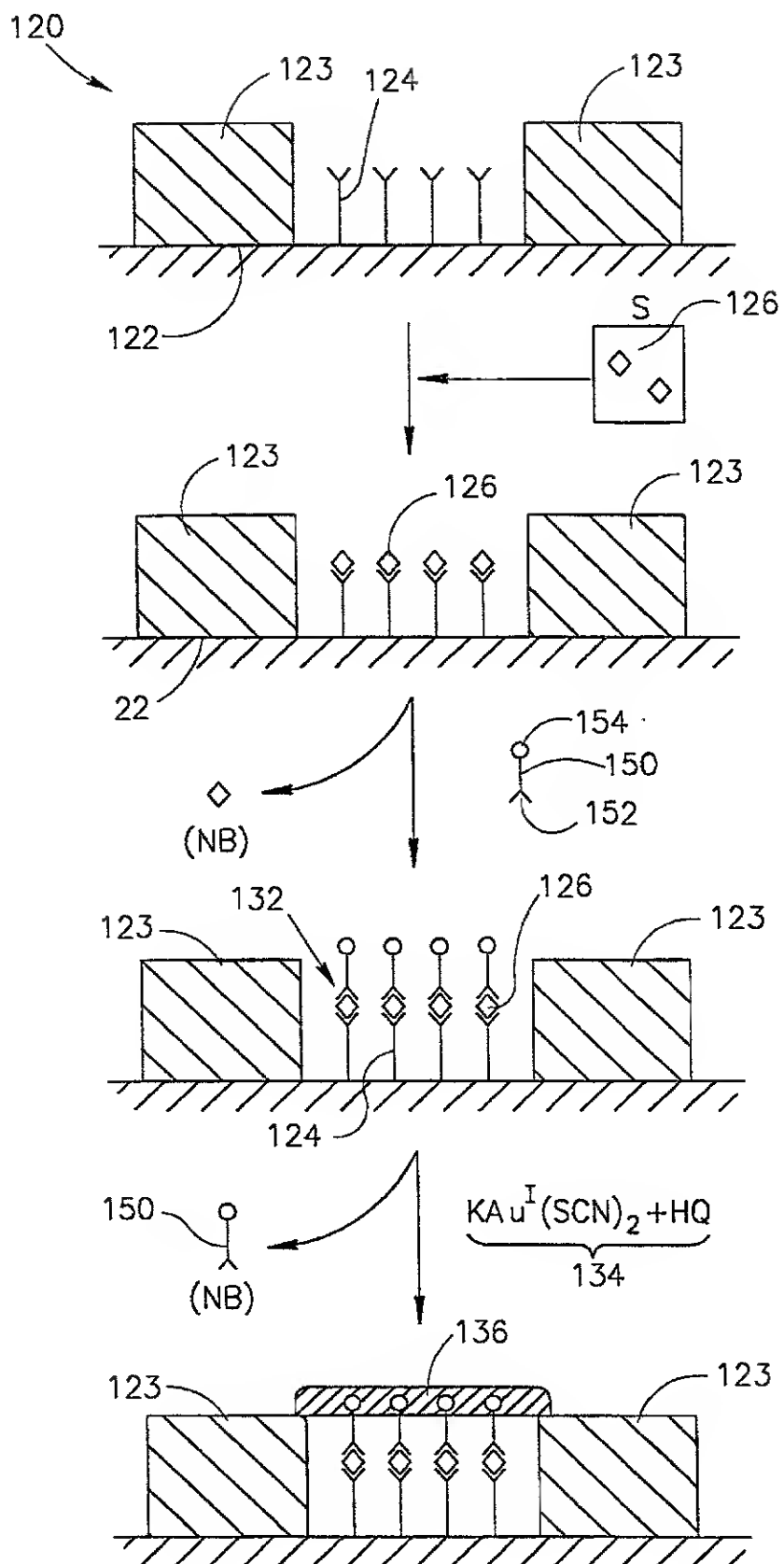


FIG. 3

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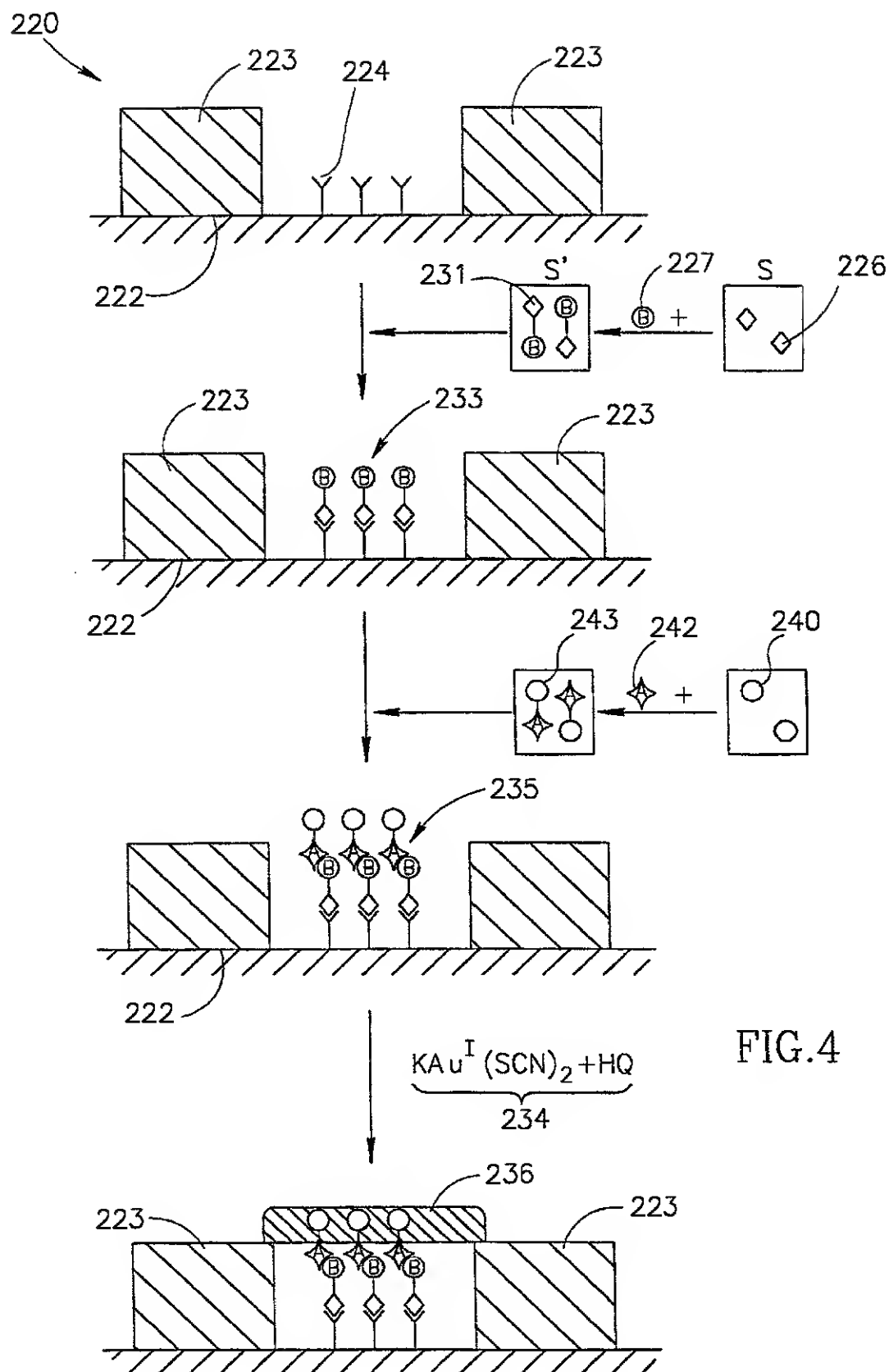


FIG.4

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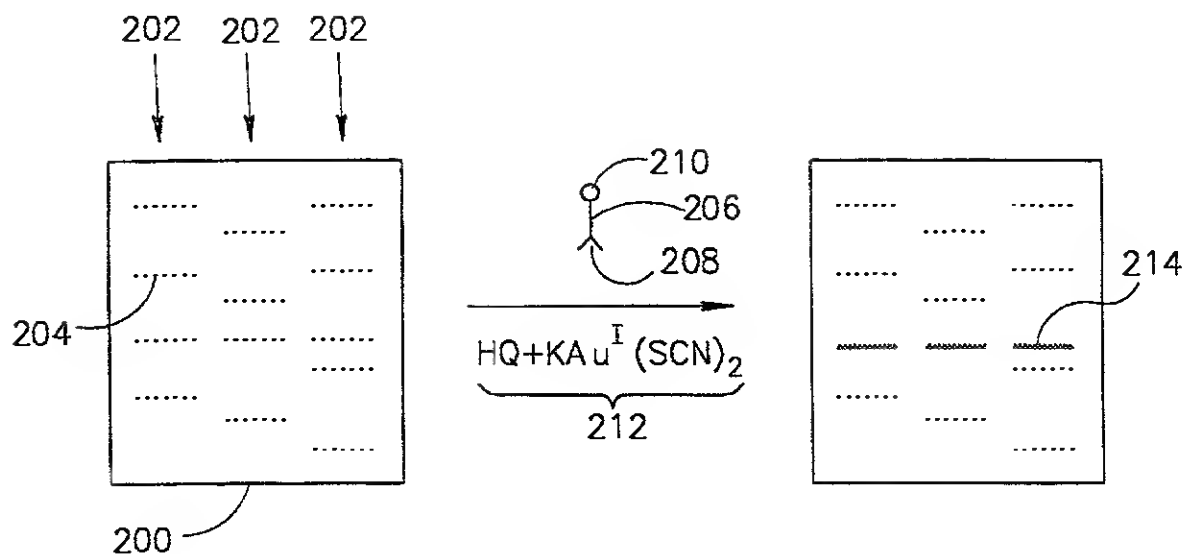


FIG. 5

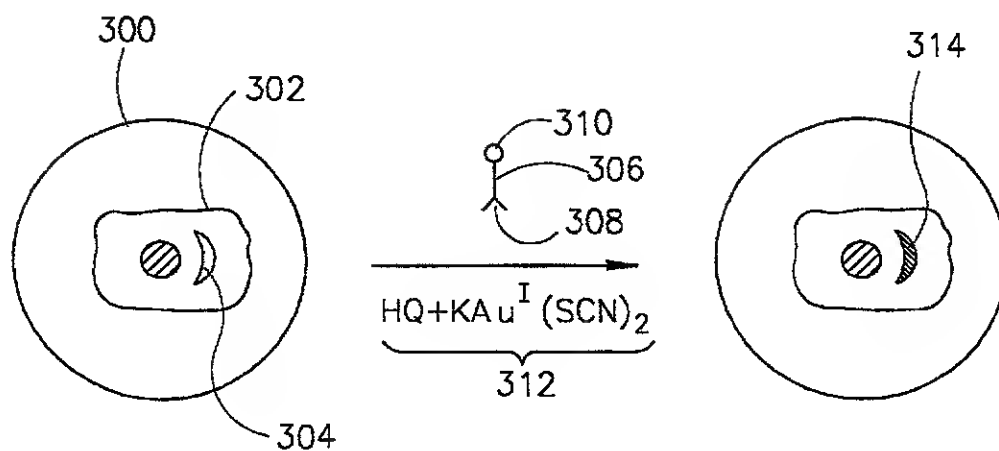


FIG. 6

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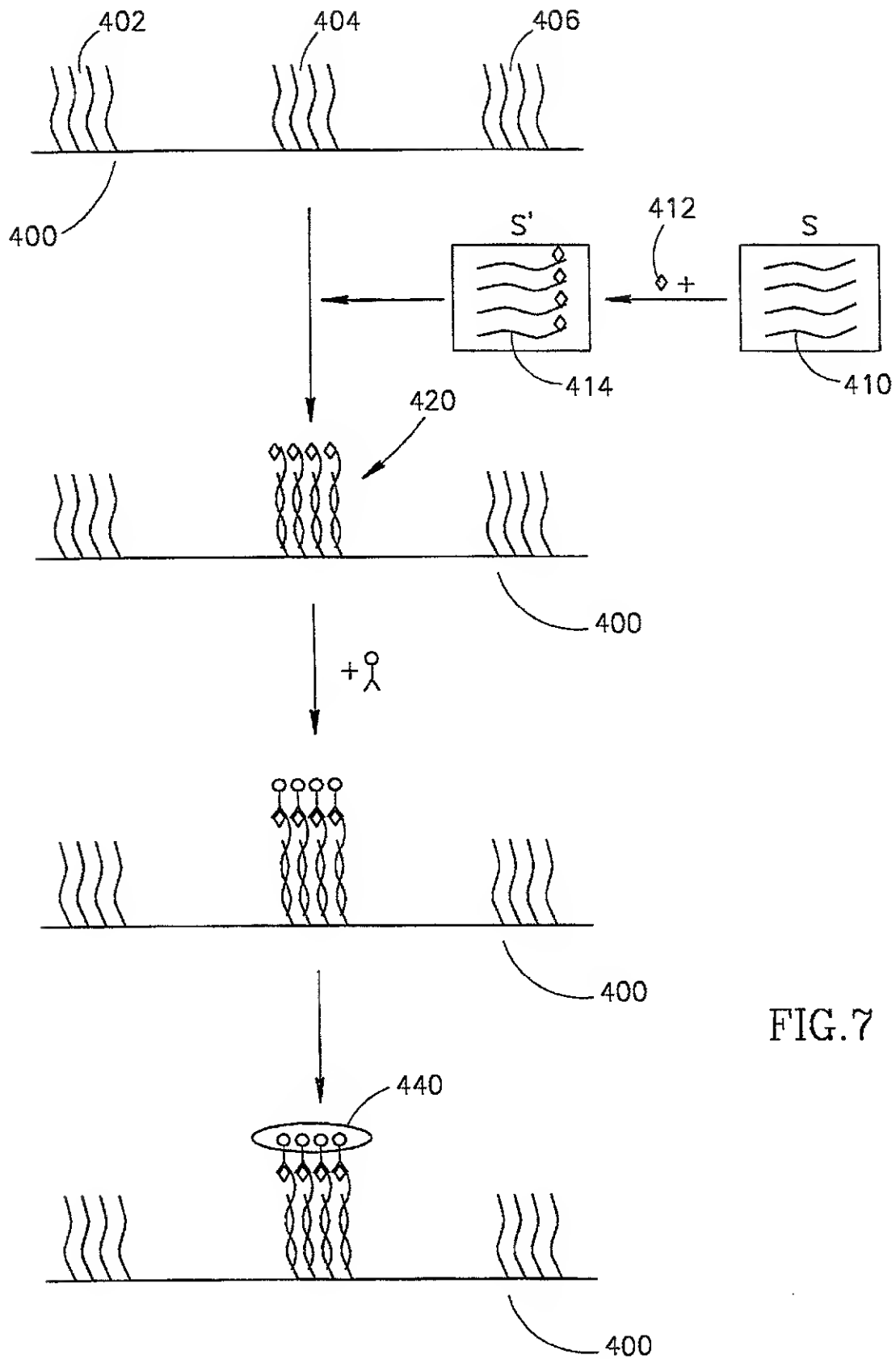


FIG. 7

IF THERE IS MORE THAN ONE INVENTOR USE PAGE Z AND PLACE AN "X" HERE ☒
(Discard this page in a sole inventor application)

2-0

1 Typewritten Full Name of Joint Inventor _____ Year _____ Middle Initial _____ Family Name EICHEN

2 Inventor's Signature: [Signature] Given Name _____ Middle Initial _____ Family Name _____

3 Date of Signature: 9 13 81 Month Day Year

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3-0

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3 Date of Signature: _____ Month _____ Day _____ Year _____

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Citizenship: _____

Post Office Address: _____
(Insert complete mailing address, including country) _____

1 Typewritten Full Name of Joint Inventor _____ Given Name _____ Middle Initial _____ Family Name _____

2 Inventor's Signature: _____

3 Date of Signature: _____ Month _____ Day _____ Year _____

Residence: _____ City _____ State or Province _____ Country _____

Citizenship: _____

Post Office Address: _____
(Insert complete mailing address, including country) _____

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1, and insert the actual date of signing on line 3.

This form may be executed only when attached to the first page of the Declaration and Power of Attorney of the application to which it pertains.